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EFFECT OF PLANT PROTEINASES ON SOME PROPERTIES OF BRINE CURED PORK

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Protein hydrolysis of muscle and connective tissue catalysed by proteinases results in physical, biochemical and structural changes of meat. These processes, affected by proteolytic enzymes present in meat, advance very slowly.

In the course of processing, however, meat may be treated by some other proteolytic enzymes, as plant proteinases - ficin, papain and bromelain, which speed up above mentioned changes in meat, especially tenderness (2,7,9,13,18).

Data in literature on plant proteinases application are generally related to beef (1,3,5,8,1o,13,15,16,17). It is reasonable because beef has much more rough structure than pork and it is very often tough after culinary and heat treatment.

Tendency toward shortening of pasteurised canned pork products processing has caused appearance of tough meat pieces.

The present work was carried out to evaluate some effects of plant proteinases on brine cured pork making efforts to have the same time ranges and temperature ones

as in production conditions. On purpose to follow enzyme effects, we chose determination of bound water content in meat, determination of protein digestion, tenderness evaluation by instrumental and organoleptical methods and histological examinations.

EXPERIMENTAL METHODS

Raw material.- M.biceps femoris and m.longissimus dorsi were obtained from ten-month-old hogs. They were cooled 24 hours at +4°C after hog slaughter and then cut in slices 3 cm thick.

pH measurements of meat extract (meat/water = 1/1) were done by means of Pye pH - meter.

Enzyme preparations.- Powdered enzymes - papain, ficin and tri-zyme (mixture of papain, ficin and bromelain) were obtained from "Heller and Co", Chicago. Enzyme preparations were added to curing brines.

Curing brine was made by adding 15 grams of $NaNO_2$, 1o grams of $NaNO_3$, 5oo grams of polyphosphates and 15o grams of sugar to 1o litres of salt solution having 18° Bé density. Besides mentioned components other curing brines contained papain, ficin of tri-zyme in concentrations of 2o grams/litre, 3o grams/litre or 5o grams/litre.

1o percents of the curing brine, in relation to the original sample weight, were injected. In one case

samples were injected by curing brine and in another one they were immersed in the curing brines for 15 minutes or 24 hours. Samples treated with the curing brine without enzymes were controls.

Injected pieces were immersed in the curing brine for 24 hours before thermal treatment. Before examination all samples were packed in Rilsan bags, vacuum sealed and cooked in water at 80°C for 2 hours.

Physical, chemical and histological examinations were done after 10 days storage at 10°C. Samples for these examinations were cut from the middle of meat pieces.

Tenderness evaluation was carried out by means of penetrometer of own construction. Slices 4 mm thick, intended for tenderness evaluation, were cut from the middle of the sample. Needle area is 10 sq.mm. Penetration force was determined at 4 mm depth. Examination results are avarage of 10 measurements.

Tenderness evaluation by palpation method was carried out by five judges.

Determination of protein digestion in controls and samples treated with enzymes was done by Gretillat's method (4).

Bound water values were obtained by subtracting the value for free-water content /determined by Grau method, modified by Sonja Karan-Djurdjić (6)/from the value for total water content (determined by drying in the oven at 105°C till the successive weighing showed no loss in weight).

Microscopic preparations were made from frozen samples (cut by freezing microtome - without previous fixing) in one case, and in another case, they were fixed by 4 percent formol and cut from the paraffin block. In both cases preparations were stained with hematoxylin and eosine by Böner method.

RESULTS AND DISCUSSION

Fig.1. illustrates significant difference in bound water content in samples of m.longissimus dorsi and m.biceps femoris injected with curing brines having different enzyme concentrations and in controls - injected with the curing brine without enzymes. Bound water content increases with enzyme concentration increase. In m.longissimus dorsi samples, the best results in bound water content were obtained by tri-zyme and in m.biceps femoris ones by papain.

From results of tests for bound water content in samples of m.longissimus dorsi and m.biceps femoris immersed for 15 minutes (Fig.2) and 24 hours (Fig.3) in the curing brine with different enzyme concentrations, there could not be remarked any regularities in bound water content in relation to particular enzyme preparation as well as to enzyme concentration.

Increase of bound water content with enzyme concentration increase in brine injected samples and irregularities in this respect in samples immersed in the curing brines for

15 minutes and 24 hours show that plant proteinases influence meat hydration but in immersed samples they could not penetrate throughout the sample and so their influence there could not be registered.

From data on protein digestion (Figs. 4, 5 and 6), it may be concluded that only samples injected with the curing brines having different enzyme concentrations compared with corresponding controls offer possibility for making conclusions on effect of papain, ficin and tri-zyme on protein digestion but for immersed samples no regularity can be remarked.

Fig.4 indicates that better protein digestion is obtained with higher enzyme concentrations. In samples of m.longissimus dorsi treated with ficin, protein digestion rate was the highest, it was lower in papain treated samples and the lowest in tri-zyme treated ones. In samples of m.biceps femoris, the best protein digestion was reached by papain, worse with tri-zyme and the worst with ficin.

From experimental data, it is evident that the desired effect, regarding protein digestion, was only reached in samples injected with the curing brine with enzymes.

Results of tenderness evaluation, presented in Figs. 7, 8 and 9, show that m.longissimus dorsi controls were more tender, as it was expected, than m.biceps femoris ones. By tenderness evaluation we got the same results as by determinations of bound water content and of protein digestion.

Namely, by samples immersion in enzyme solutions, besides prolongation of treatment time (24 hours), it is not possible to achieve uniform tenderization throughout the meat sample.

Injecting curing brines with enzymes greatly increases meat tenderness. As concentration of injected enzymes is higher, meat is more tender. Our studies showed, using panel scoring methods, that the range of optimal tenderness was from 1,oo to 1,5o kilogram (penetration force). The Fig.7 illustrates that tenderness values for m.longissimus dorsi controls were very close to optimal values and so the desired effect was obtained already with enzyme concentrations of o,2 and o,3 percent. The treatment of m.longissimus dorsi samples with enzymes in concentration of o,5 percent showed to be undesirable because it resulted in excessive meat tenderization.

All m.biceps femoris samples were remarkably tougher than m.longissimus dorsi ones and therefore the higher enzyme concentration was needed for their tenderization, that is o,5 percent enzyme concentration. Enzyme concentration of o,3 percent showed to be satisfactory only for samples treated with tri-zyme.

Results obtained with instrumental methods were, in general, in correlation with organoleptic examinations, that is with palpation method (Fig.1o). Evaluation was done with six scores. Optimum tenderness was from 3 to 4; scores less than 3 indicate less tender samples and over 4-too tender samples, muscle fibers separated under fingers.

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Histological examinations were carried out only with brine injected samples in which after enzyme application, changes of chemical and physical character were remarked.

By microscopic examinations (Fig.11) we obtained nearly identical results as Wang (15,16,17). There are not great differences in effect of particular enzymes. Tri-zyme affects the loss of cross striations if it is used in very high quantities. Compared with tri-zyme ficin less and papain least affect disappearance of cross striations. Of three tested plant proteinase preparations papain has the highest effect on sarcolemma disintegration, plasm separation from the membrane and collection of ditritus mass in intracellular spaces.

Possibility of remarking described microscopic changes is especially dependent on histological techniques. Therefore, we consider that in this moment the attention has to be paid on evaluation of results of microscopic examinations. Numerous changes in structure of muscle tissue described by many authors are in good deal the artifact of preparation techniques.

As proteinases action is in relation to pH values of the medium, it has to be mentioned that the avarage pH value of m.longissimus dorsi before brine curing was 5.43 and of m.biceps femoris 5.91.

Great differences in results of chemical and physical examinations between controls and brine injected samples

indicate significant effect of enzymes as catalysts in hydrolysis of peptide chains - proteolysis. All samples injected with enzymes have the higher bound water percentage, better protein digestion and they are more tender. There is the significant difference in these characteristics between samples of the same muscle treated with various enzyme concentrations. In injected samples increase of enzyme concentration resulted in increase of bound water content as well as in promotion of tenderness. This correlation, however, does not exist in samples immersed in curing brines having various enzyme concentrations. Irregularities in results of bound water measurements, determination of protein digestion and evaluation of tenderness in immersed samples indicate very bad enzyme penetration and in the same time inconvenience of such method of enzyme application.

Differences in action of papain, ficin and tri-zyme on protein changes in injected samples may be explained by their specific action and not uniform distribution throughout the muscle tissue and connective one.

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On the base of obtained results it may be concluded that plant proteinases affect better protein digestion and better meat hydration. Although pork is more tender than beef, pork treatment by plant proteinases results in more desirable tenderness.

All these conclusions could be done only with injected samples. Immersed samples indicate that immersion, as a treatment method, is inconvenient for this purpose.

Histological techniques are the most important for remarking structural changes in enzyme treated samples.

EFFECT OF PLANT PROTEINASES ON SOME PROPERTIES OF BRINE CURED PORK

Summary

The purpose of the study was to examine application of plant proteinases - papain, ficin and tri-zyme (mixture of papain, ficin and bromelain) - in brine cured pork making efforts to process meat in time ranges and temperature ones that correspond to conditions in canned pasteurized meat products processing. For following the effect of these enzymes, determination of protein digestion and bound water content, evaluation of tenderness by instrumental and panel scoring and histological examinations were chosen.

On the base of obtained results, it may be concluded that plant proteinases affect better protein digestion and better meat hydration. Although pork is more tender than beef, pork treatment by plant proteinases results in more desirable tenderness.

All these conclusions could be done only with injected samples. Immersed samples indicate that immersion, as a treatment method, is inconvenient for this purpose.

Histological techniques are the most important for remarking structural changes in enzyme treated samples.

ВЛИЯНИЕ РАСТИТЕЛЬНЫХ ПРОТЕОЛИТИЧЕСКИХ ФЕРМЕНТОВ НА НЕКОТОРЫЕ СВОЙСТВА СОЛЕНОЙ СВИНИНЫ

Р Е З Ю М Е

Целью исследования было изучение результатов применения растительных протеолитических ферментов - папаина, фицина и трисима (смеси папаина, фицина и бромелина) в соленой свинине, причем стремление было таково, чтобы его обработка как по времени, так и по температурному режиму поддерживалась в условиях, соответствующих производству полуконсервов. Для наблюдения за действиями данных ферментов выбрано определение усвояемости белков и количества связанной в мясе воды, оценки консистенции инструментальными и органолептическими методами и гистологические исследования.

На основании достигнутых результатов проистекает, что применением растительных протеолитических ферментов в соленой свинине достигается гораздо лучшая усвояемость протеина и лучшая гидратация мяса. Хотя свинина более нежной консистенции чем говядина, ее обработкой растительными протеолитическими ферментами достигается еще более желательная нежная консистенция.

Эти выводы могут быть сделаны лишь для шприцованных образцов. Образцы, выдерживавшиеся в посолочной смеси указывают, что данный способ, как метод обработки, непригоден для данной цели.

Гистологические техники являются самими важными для того, чтобы можно было заметить структурные изменения в образцах обработанных ферментами.

INFLUENCE DES PROTEASES VEGETALES SUR CERTAINES QUALITES DE LA VIANDE DE PORC SALAISONNE

Résumé

Le but des examens a été de voir quels seront les résultats de l'application des protéases végétales - papaine, fitine et trisine (mélange de Papaïne, fitine et bromeline) - sur la viande de porc de la salaison, en s'efforcant de garder son traitement dans les limites de temps et de température correspondant aux conditions de la production des semi-conserves. Afin de suivre ces enzimes on a choisi la détermination de la digestibilité des protéines et les quantités d'eau liée dans la viande, l'estimation de la consistance étant faite par des méthodes instrumentales et organoléptiques et les examens histologiques.

Le résultats obtenus démontrent que l'application des protéases végétales sur la viande de porc de salaison permet une digestibilité plus grande des protéines beaucoup plus grand et une hydration de la viande meilleure. La viande de porc est de consistance plus tendre que celle du boeuf, mais le traitement de la viande de porc par des protéases végétales résulte en une consistance plus désirable est plus tendre.

Ces conclusions ne peuvent être valables que pour des échantillons injectés. Les échantillons trempés prouvent que l'immersion, en tant que méthode de traitement, se révèle incompatible.

Les techniques histologiques sont les plus importantes pour la détermination des changements structuraux dans les échantillons traités par les enzimes.

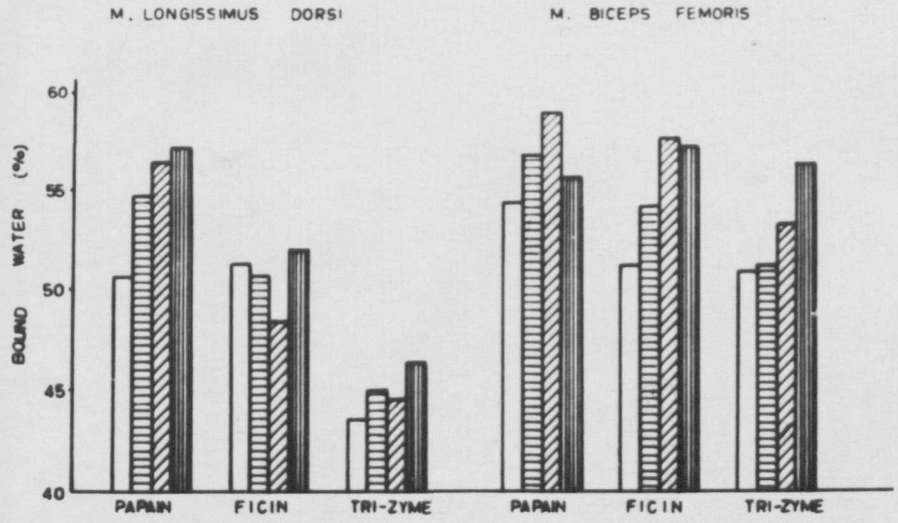


FIG. 3. PERCENTAGE OF BOUND WATER IN SAMPLES OF M. LONGISSIMUS DORSI AND M. BICEPS FEMORIS IMMERSED 24 HOURS IN CURING BRINES HAVING DIFFERENT ENZYME CONCENTRATIONS

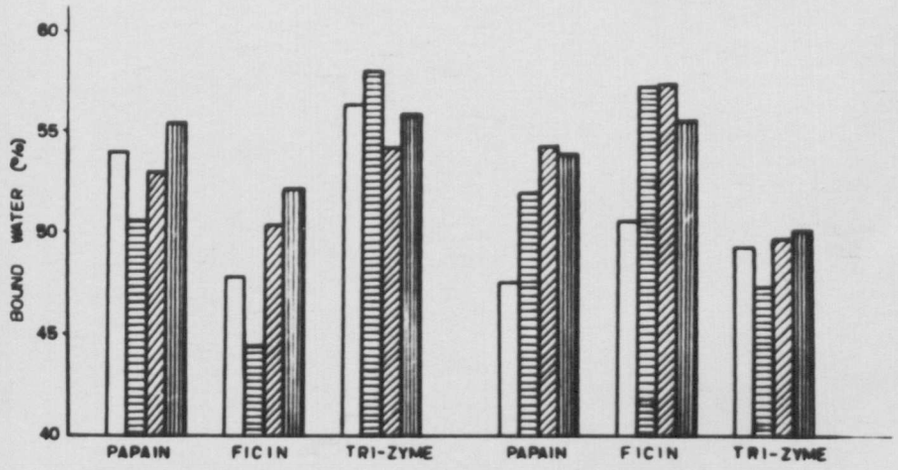


FIG. 2. PERCENTAGE OF BOUND WATER IN SAMPLES OF M. LONGISSIMUS DORSI AND M. BICEPS FEMORIS IMMERSED 15 MINUTES IN CURING BRINES HAVING DIFFERENT ENZYME CONCENTRATIONS

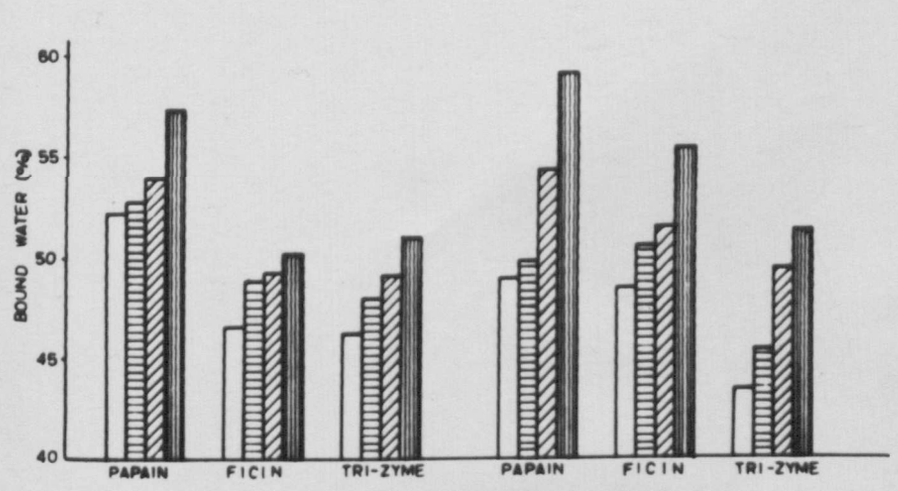
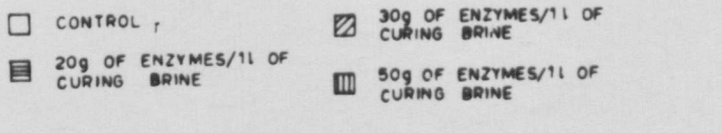


FIG. 1. PERCENTAGE OF BOUND WATER IN SAMPLES OF M. LONGISSIMMUS DORSI AND M. BICEPS FEMORIS INJECTED WITH DIFFERENT ENZYME CONCENTRATIONS



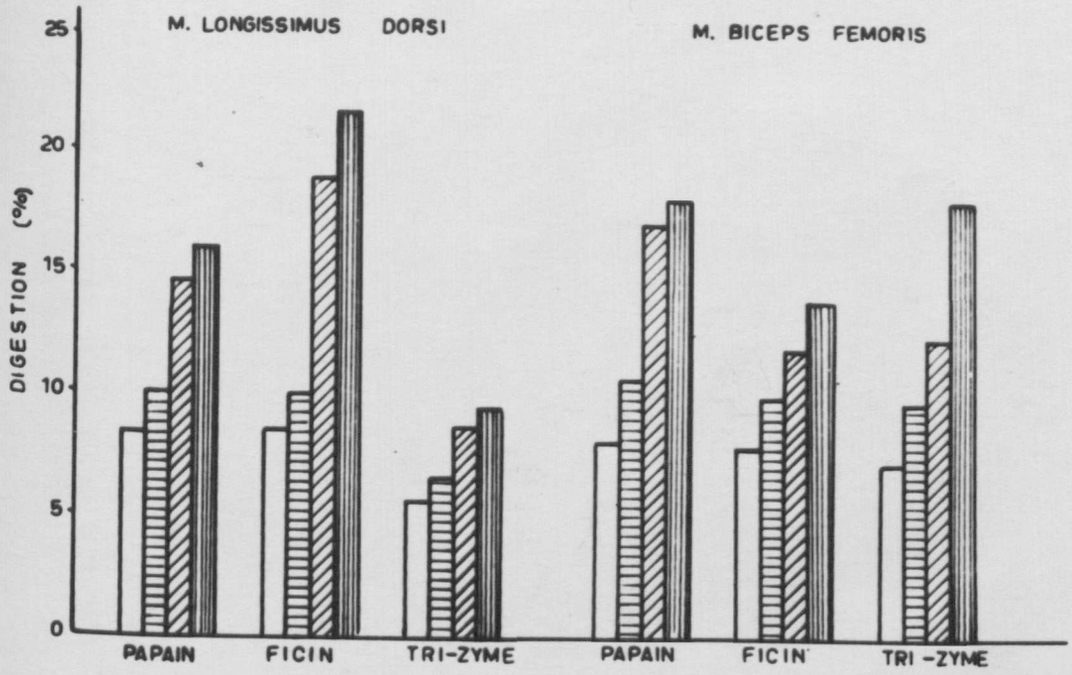


FIG. 4. PROTEIN DEGESTION IN SAMPLES OF M. LONGISSIMUS DORSI AND M. BICEPS FEMORIS INJECTED WITH CURING BRINES HAVING DIFFERENT ENZYME CONCENTRATIONS

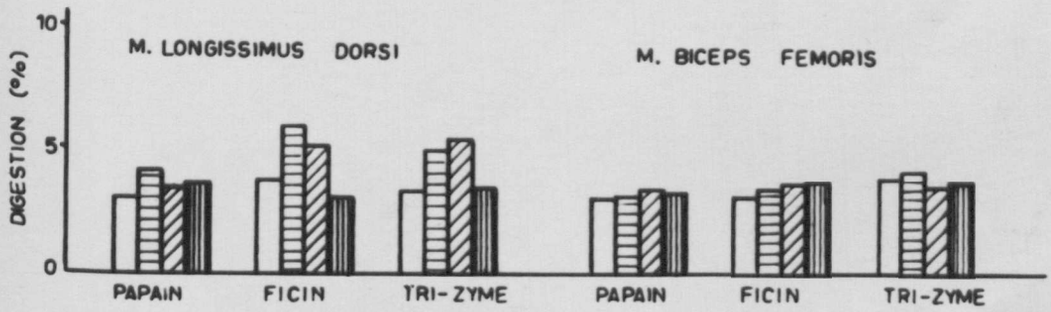


FIG. 5. PROTEIN DIGESTION IN SAMPLES OF M. LONGISSIMUS DORSI AND M. BICEPS FEMORIS IMMERSED 15 MINUTES IN CURING BRINES HAVING DIFFERENT ENZYME CONCENTRATIONS

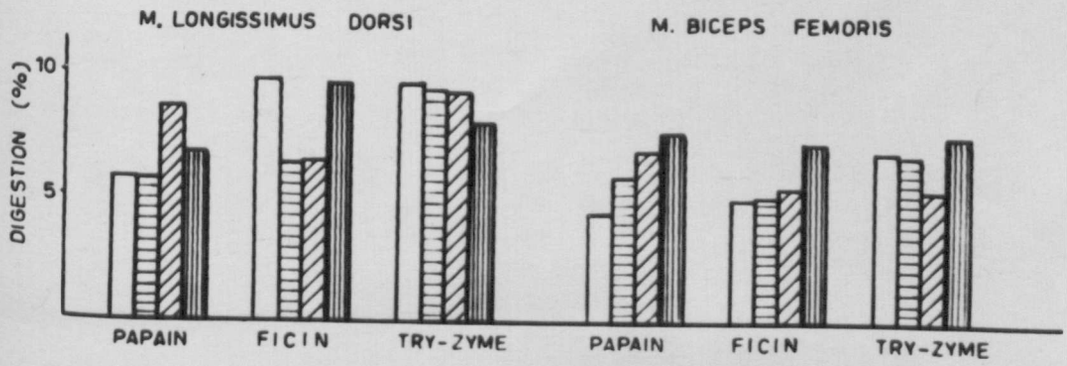
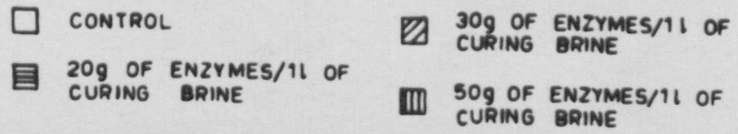


FIG. 6. PROTEIN DIGESTION IN SAMPLES OF M. LONGISSIMUS DORSI AND M. BICEPS FEMORIS IMMERSED 24 HOURS IN CURING BRINES HAVING DIFFERENT ENZYME CONCENTRATIONS



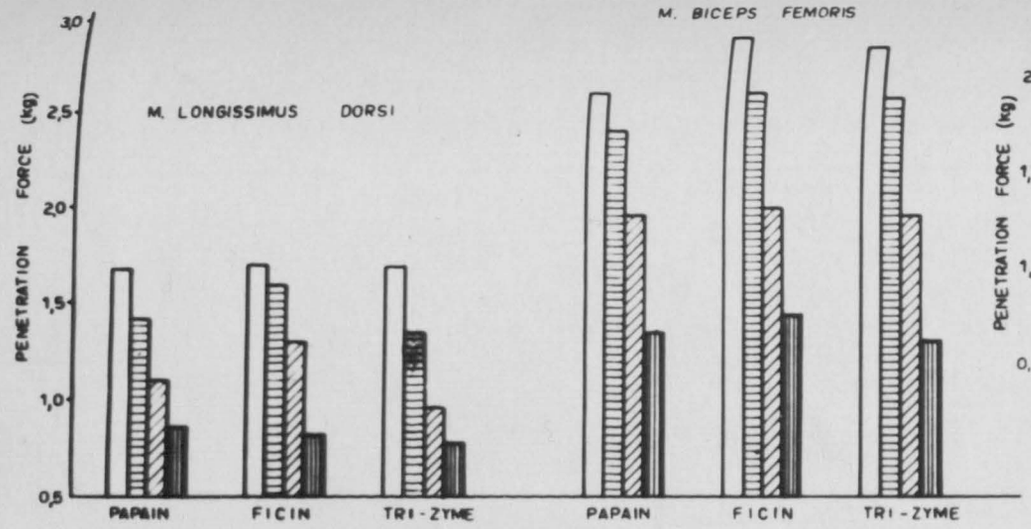


FIG. 7. TENDERNESS OF SAMPLES OF M. LONGISSIMUS DORSI AND M. BICEPS FEMORIS, INJECTED WITH CURING BRINES HAVING DIFFERENT ENZYME CONCENTRATIONS, DETERMINED BY PENETROMETER

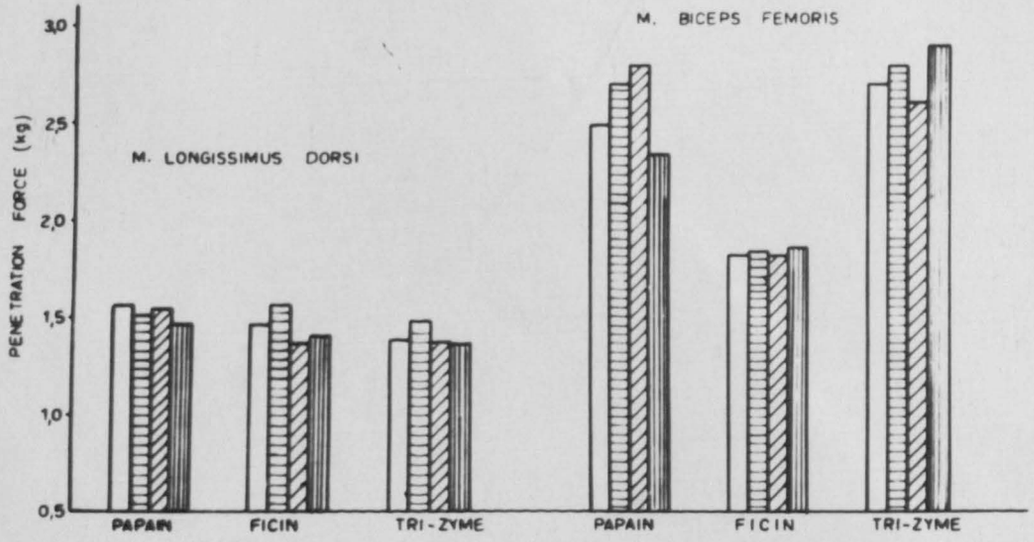


FIG. 8. TENDERNESS OF SAMPLES OF M. LONGISSIMUS DORSI AND M. BICEPS FEMORIS, IMMERSED 15 MINUTES IN CURING BRINES HAVING DIFFERENT ENZYME CONCENTRATIONS, DETERMINED BY PENETROMETER

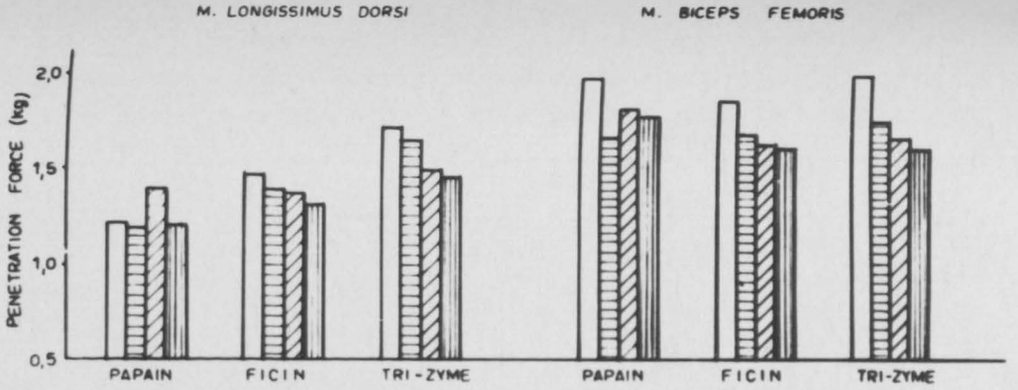


FIG. 9. TENDERNESS OF SAMPLES OF M. LONGISSIMUS DORSI AND M. BICEPS FEMORIS, INJECTED WITH CURING BRINES HAVING DIFFERENT ENZYME CONCENTRATIONS, DETERMINED BY PENETROMETER

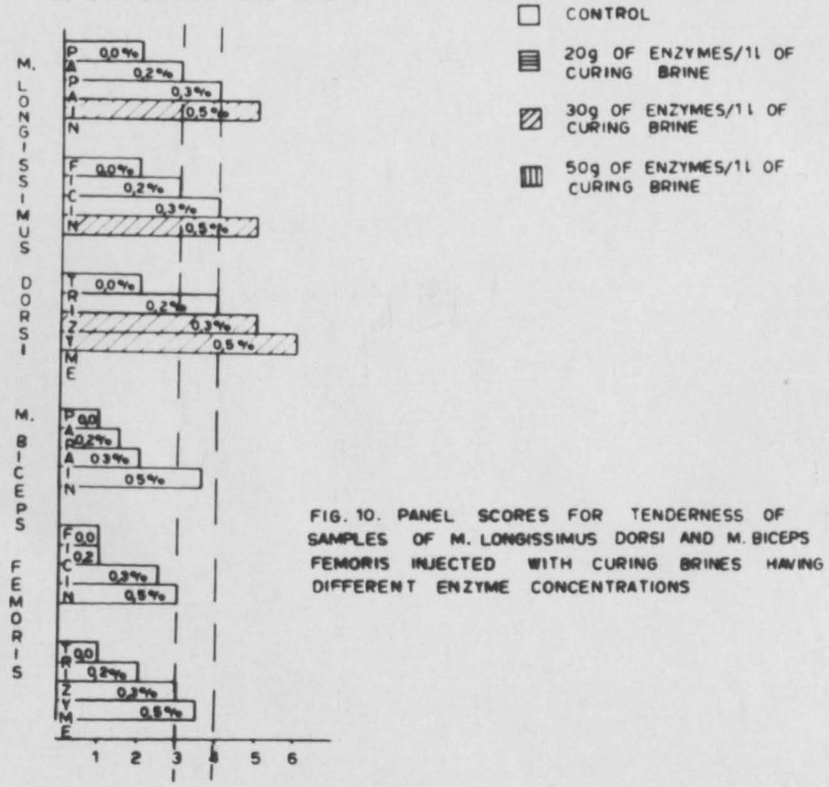
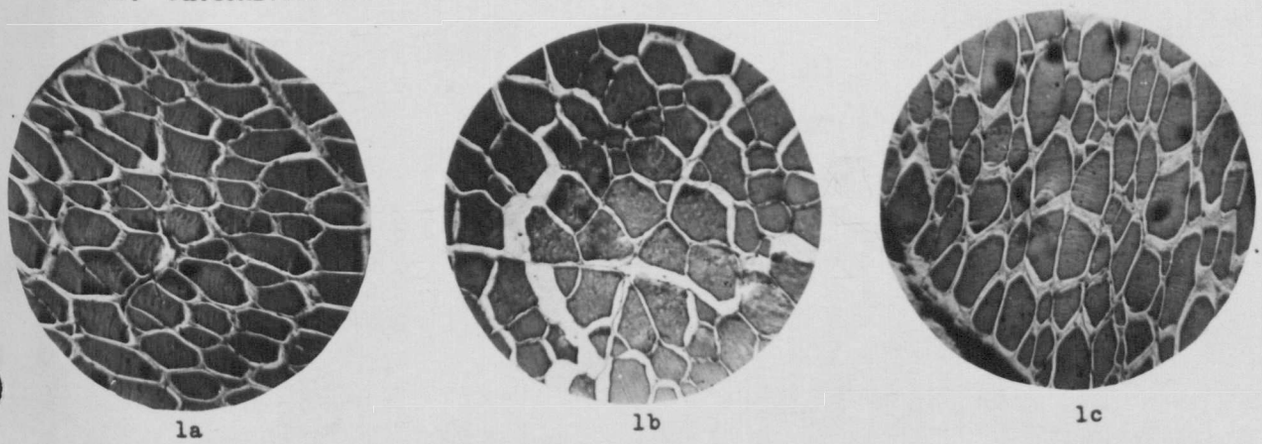


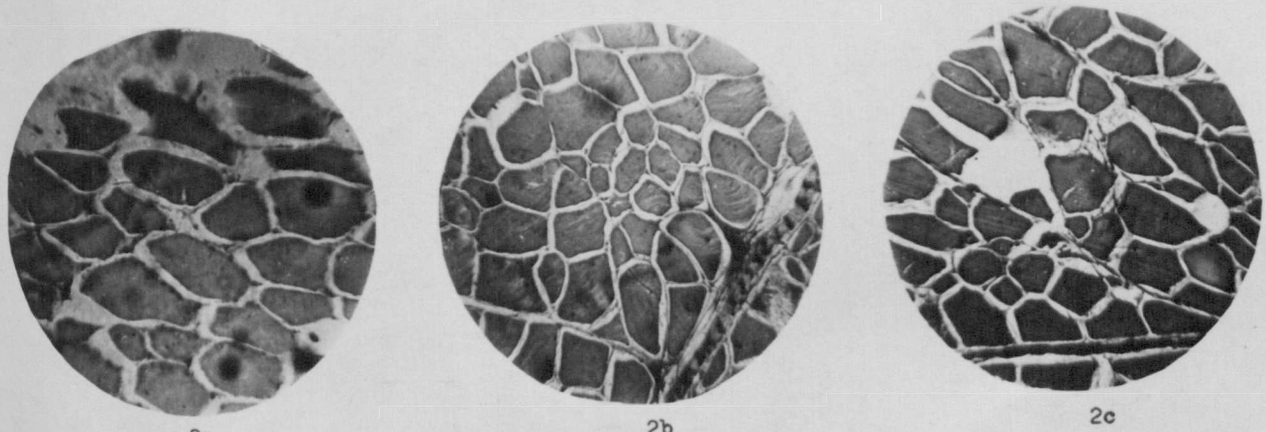
FIG. 10. PANEL SCORES FOR TENDERNESS OF SAMPLES OF M. LONGISSIMUS DORSI AND M. BICEPS FEMORIS INJECTED WITH CURING BRINES HAVING DIFFERENT ENZYME CONCENTRATIONS

FIG. 11. PHOTOMICROGRAPHS MADE FROM SECTIONS OF INJECTED SAMPLES WITH ENZYMES



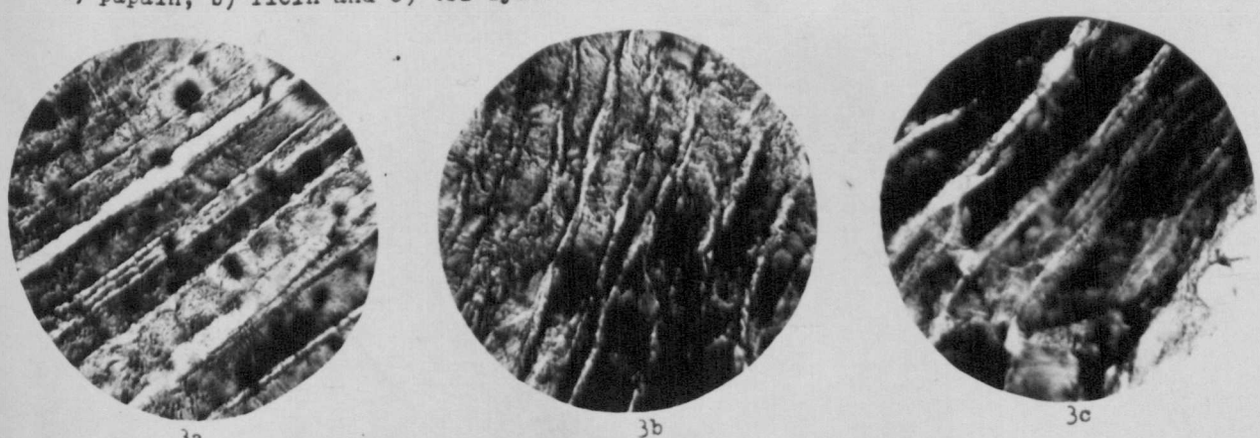
1a 1b 1c

1. Cross sections of m. longissimus dorsi samples treated with the highest contentrations (o,5%) of a) papain b) ficin and c) tri-zyme.



2a 2b 2c

2. Cross sections of m. biceps femoris treated with the highest concentrations (o,5%) of a) papain, b) ficin and c) tri-zyme.



3a 3b 3c

3. Longitudinal sections of treated muscles - unfixed preparations - injected with higher concentrations (o,3%) of a) papain (longissimus dorsi) b) ficin and c) tri-zyme (m. biceps femoris).

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